targeted therapies are thus needed to improve outcomes and immunotherapy has the potentials to fulfill this need. We have previously shown that genetically modified T cells expressing a chimeric antigen receptor (CAR) composed of NKG2D and the CD3-ζ chain have potent antitumor activity against several adult malignancies. Since at present only limited targets are available for the immunotherapy of osteosarcoma, the aim of this study was to determine if NKG2D-ligands (NKG2D-L) are expressed on the cell surface of osteosarcoma and if NKG2D-L-specific T cells recognize and kill osteosarcoma.

Methods and Results: NKG2D-L expression was determined by FACS analysis as well as RT-PCR. 7 out of 8 cell lines tested expressed one or several NKG2D-L on their cell surface. NKG2D-L-specific T cells were generated by transducing CD3/CD28-activated T cells with a SFG retroviral vector encoding the NKG2D-L-specific CAR and truncated CD19 to allow detection of transduced T cells. NKG2D-L-specific T cells from healthy donors produced IFN-γ in contrast to mock transduced T cells after coculture with NKG2D-L-positive osteosarcoma cells. In cytotoxicity assays, NKG2D-L-specific T cells killed NKG2D-L-positive osteosarcoma cells where as activated, autologous lymphocytes and NKG2D-L-negatvie osteosarcoma cells were not killed.

Conclusion: We have shown that NKG2D ligands are expressed on osteosarcoma and that NKG2D-L specific T cells recognize and kill osteosarcoma. Murine xenograft studies are in progress to confirm these findings in vivo. Adoptive immunotherapy with NKG2D-L-specific T cells may represent a promising immunotherapeutic approach for osteosarcoma.

SOLID TUMORS

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CCR2B-TRANSDUCED CYTOTOXIC T LYMPHOCYTES SHOW ENHANCED HOMING *IN VIVO* TOWARD CCL2-SECRETING NEUROBLASTOMA: IMPLICATIONS FOR ENHANCED ADOPTIVE IMMUNOTHERAPY

Craddock, J.A., Lu, A., Rooney, C.M., Foster, A.E. Baylor College of Medicine, Houston, TX

The chemokine CCL2 (MCP-1) is produced by a variety of cancers including neuroblastoma, melanoma, breast cancer and hematological malignancies. However, cytotoxic T lymphocytes (CTL) used in clinical trials to treat these diseases do not express CCR2, the receptor for CCL2. This deficiency may contribute to suboptimal trafficking to tumor sites and reduced clinical efficacy. Therefore, we examined whether genetically modifying CTL with CCR2 could increase their homing towards tumor cells that produced CCL2. CTL were first examined by flow cytometry for chemokine receptor expression using antibodies to CCR2, CCR4, CCR5, CCR7 and CXCR4. These CTL showed nominal expression of CCR2 (<1%), low expression of CCR4 (10%), CXCR4 (25%), CCR7 (5%), and high expression of CCR5 (70%). ELISA for CCL2 was performed on established neuroblastoma cell lines (IMR-32, LAN-1, SK-N-SH, JF) and on neuroblastoma cell lines derived from patients primary tumors. All neuroblastoma cell lines secreted CCL2 in variation but notably, all patient-derived primary tumor cell lines secreted high levels of CCL2. T cells were activated by anti-CD3/anti-CD28 antibody stimulation for 3 days in the presence of IL-2, and then transduced using a CCR2b retroviral vector. Expression of CCR2b was detected by flow cytometry producing up to 78% transduction. *In vitro* chemotaxis of CCR2b-transduced T cells showed enhance migration to soluble CCL2 (>3 fold) as well as to supernatants from CCL2-secreting cell lines (>2 fold) compared to non-transduced T cells. To study *in vivo* migration, we co-transduced T cells with EGFPluciferase and CCR2b. Subsequent tail vein infusion of these co-transduced T cells into mice bearing CCL2 secreting tumors (SK-N-SH) showed preferential migration of only CCR2b-transduced T cells toward the tumor site when compared to non-transduced T cells. These results demonstrate the feasibility of influencing the trafficking of adoptively transferred T cells toward CCL2 secreting neuroblastoma and thereby suggesting a potential means to enhance future adoptive immunotherapies targeting neuroblastoma.

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HIGH DOSE CHEMOTHERAPY WITH BLOOD OR MARROW TRANSPLANT FOR RHABDOMYOSARCOMA: A CIBMTR ANALYSIS

Stiff, P., Agovi, M.-A., Antman, K., Camitta, B., Cairo, M., Childs, R., Edwards, J.R., Gale, R.P., Hale, G., Horowitz, M.M., Lazarus, H., Arora, M. CIBMTR, Milwaukee, WI

Rhabdomyosarcoma, the commonest soft tissue sarcoma in children is initially chemosensitive with cures in 70%, but 5 year survival for relapsed patients is 15–20% and only 5% for those with alveolar/undifferentiated subtypes. Since autologous stem cell transplantation (ASCT) is not clearly defined for this disease, we describe outcomes of 62 patients who received ASCT from 1989–2003 and reported to CIBMTR. Pathology was confirmed through review of reports for all cases. Transplant related mortality (TRM), progression-free survival (PFS) and overall survival (OS) were evaluated. We also compared subgroups: poor risk histology (alveolar/undifferentiated, n = 34) vs good risk (embryonal/botryoid, n = 21); patients in 1st remission (REM1, n = 38) vs patients who relapsed (REL, n = 21); those presenting with metastases (n = 39) vs those without (n = 19); patients with poor risk histology who were in REM1 (n = 20) vs REL (n = 12) and those with good risk histology who were in REM1 (n = 13) vs REL (n = 8). For the entire cohort, 73% were <20 yrs in age, 52% were male, 39% presented with bulk >5cm, and 63% had metastasis at diagnosis. Fifty percent had alveolar histology, 32% embryonal subtype, 67% were in REM1, and 92% were chemosensitive at ASCT. The conditioning regimens included melphalan in 53% and etoposide in 67%. There were no differences in the remission status at ASCT (63% vs 62% in REM1; p = 0.9) or median time from diagnosis to ASCT (p = 0.7) for those with good vs poor risk histology. Patients with poor risk histology were more likely to have marrow involved at diagnosis (44 vs 14%;p = 0.2), a CR to primary therapy (53% vs 26%;p = 0.1) and being in a CR at ASCT (70% vs 48%;p = 0.2). For the entire group, the TRM at 1 year was 5% (95% CI, 1–12); the 5 year PFS and OS were 29% (95% CI, 18–41) and 32%(95% CI, 21–44) respectively. There were no significant difference in 5 year PFS and OS between: those with poor risk vs good risk histology (p = 0.6;p = 0.9), those transplanted in REM1 vs REL (36% vs 29%;p = 0.5), those with poor risk histology transplanted in REM1 vs REL (34% vs 33%;p = 0.9), or those with good risk histology transplanted in REM1 vs REL (38% vs 25%;p = 0.2). These data indicate that ASCT for this disease are typically done for patients with chemosensitive disease and suggest that when performed after relapse, long-term survival is seen in approximately 1/3, seemingly better than previous reports of standard dose salvage for those with alveolar/undifferentiated histologies.

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DEPLETION OF CD4 T CELLS LEADS TO INCREASED TUMOR IMMUNITY AFTER HSCT IMMUNOTHERAPY BUT RESULTS IN LOSS OF MEMORY

Jing, W., Johnson, B.D. Medical College of Wisconsin, Milwaukee, WI

High-risk neuroblastoma is a clinically challenging disease. We have demonstrated that established neuroblastoma can be eliminated in a murine model by using a multi-faceted immunotherapeutic approach involving syngeneic hematopoietic stem cell transplantation (HSCT), T cell transfer (cellular immunotherapy), and tumor vaccination. One surprising finding in these experiments was that while depletion of CD4 T cells resulted in decreased anti-tumor immunity in mice given naïve T cells as cellular immunotherapy, if the transferred T cells were pre-sensitized to tumor antigens (i.e., from tumor-vaccinated donors) CD4 depletion resulted in increased anti-tumor immunity. The increased anti-tumor efficacy in these CD4-depleted hosts was accompanied by: (a) more vigorous proliferation of CD8 cells in the spleens and tumor-draining lymph nodes as observed in CFSE-labeling studies, (b) elevated percentages of CD8 T cells with a CD62L-CD44+ effector phenotype, (c) elevated frequencies and absolute numbers of splenic tumor-reactive (IFN-γ-producing) CD8 cells, and (d) more robust tumor infiltration by CD8 cells. However, development of long-term tumor immunity (memory) was severely compromised in these CD4-depleted hosts, as reflected by inability to resist a tumor re-challenge and diminished in vitro CD8 recall responses. This loss of memory occurred even though the adoptively transferred CD8 T cells were from donors that had been vaccinated in the presence of CD4 cells, indicating

that the continued presence of CD4 T cells after HSCT is required for the development of CD8 memory. Upon investigating the mechanism(s) of memory loss, we observed an accelerated contraction of tumor-reactive CD8 cells in the CD4-depleted mice. This accelerated loss of tumor-reactive CD8 cells correlated with increased percentages of annexin-V+ pre-apoptotic CD8 cells during the contraction phase (day 21 after HSCT). A significant proportion of the annexin-V+ CD8 cells co-expressed programmed death receptor-1 (PD-1), had an effector phenotype (CD62L-CD44+), and expressed diminished levels of the receptors for IL-7 and IL-15. These data suggest that PD-1/PD-L1 interactions may play a role in the loss of CD8 memory. Together, these results show that CD4+ cells can both promote and inhibit anti-tumor responses. These observations should help us design immunotherapeutic approaches that can generate "optimal" acute anti-tumor reactivity after HSCT with development of long-term immunity.

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LONG-TERM FOLLOW-UP OF METASTATIC RENAL CANCER PATIENTS UNDERGOING REDUCED-INTENSITY ALLOGRAFTING

Bregni, M., Bernardi, M., Pescarollo, A., Crocchiolo, R., Treppiedi, E., Ciceri, F., Corradini, P., Peccatori, J. Ospedale San Giuseppe, Milano, Italy; San Raffaele Scientific Institute, Milano, Italy; Ospedale Evangelico, Genova, Italy; Istituto Nazionale Tumori, Milano, Italy

Stem cell transplantation from a HLA-compatible sibling donor is an adoptive immunotherapy for cytokine-resistant, metastatic renal cancer (RCC). However, the recent introduction of several targeted therapy compounds has reduced the interest in this therapeutic strategy. We have reanalyzed our transplant series with the aim to detect long-term benefit form allografting. From February 1999 to May 2005, 25 patients with cytokine-refractory RCC received a reduced-intensity allograft from an HLA-id sibling donor. Median age was 53 years; most (24) had clear-cell histology. Median number of previous treatments was 1 (0–3). Median days from diagnosis to allograft were 822. All patients received a thiotepa, fludarabine, and cyclophosphamide conditioning regimen, and a cyclosporine-based GVHD prophylaxis. Six patients received DLI at escalating doses for progressing or non-responding disease. One-year-OS was 48% (95% CI: 28–68), and 3y-OS was 20% (95% CI: 4–36). At a median observation time of 65 months, 5 patients are alive, one in CR, one in VGPR, and three with stable disease. We have analyzed the correlation of the following variables with survival: age at transplant, time from diagnosis to transplant, serum calcium corrected for albumin levels, lactate dehydrogenase (LDH), C-reactive protein (CRP), haemoglobin level before transplantation, Karnofsky performance status, number of CD34+/kg infused, number of CD3+/kg infused, progressive disease at +90 after transplant, occurrence of acute (any grade) or chronic GvHD. At multivariate analysis, CRP value before transplant, number of CD34+ infused cells and disease status at +90 significantly correlated with survival. Survival of patients at favourable/intermediate-risk according to the MSKCC score that underwent allografting was better in comparison to the survival predicted by historical controls. We conclude that transplantation is able to induce long-term disease control in twenty percent of cytokine-refractory RCC patients. It is unknown if relapse or PD after targeted therapy will be susceptible to allograft-mediated GVT effect. The place of allografting in the treatment of metastatic RCC, alone or in combination with targeted therapies, needs reappraisal.

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LOW MORBIDITY AND MORTALITY IN A PILOT STUDY OF BUSULFAN, MELPHALAN AND TOPOTECAN AS PREPARATIVE REGIMEN FOLLOWED BY AUTOLOGOUS HEMATOPOETIC STEM CELL TRANSPLANTATION IN PEDIATRIC PATIENTS WITH HIGH RISK SOLID TUMORS (PRELIMINARY RESULTS)

Bolotin, E., Pawlowska, A., Hitt, D., Andersen, C., Shakhnovitz, M., Sato, J., Forman, S., Rosenthal, J. City of Hope, Duarte, CA

Autologous hematopoetic stem cell transplantation (HDT) has been utilized as consolidative therapy for pediatric patients with some encouraging improvement in survival. We hypothesize that by intensifying therapy which poor prognosis patients currently receive, it is possible to reduce the risk of relapse after HDT and the morbidity and mortality will be acceptable. Phase I pediatric studies of topotecan demonstrated activity against solid tumors. The dose-limiting toxicity of Phase I studies has been hematopoietic suppression. The use of hematopoietic stem cell support following a topotecan-containing preparative regimen will allow utilization of topotecan in combination with established chemotherapeutic agents. Patients were assigned to receive topotecan at 2 mg/m2 continuous infusion in combination with fixed doses of busulfan and melphalan (B-M-T). In this study, the primary outcome variable is to evaluate the toxicity of combined HDT with B-M-T. Secondary objective is to analyze the engraftment and outcomes.

Results: Thirteen patients age 2–18 year old (median = 5.4) were treated on B-M-T-protocol. Seven patients were diagnosed with neuroblastoma (NBL), Wilms tumor (n = 2), PNET (n = 2), -rhabdomyosarcoma (RBS) and Ewing sarcoma (each n = 1). Eight patients were in CR, five in PR. There were no deaths in the first100 day post HDT. There was 1 admission (1day) to ICU for observation only. Eleven patients developed grade 1–2 and 2 patients grade 3 liver toxicity (transaminitis and bilirubin elevation), all patients developed grade 2- 3 mucositis, five patients had grade 2 diarrhea, one patient developed grade 3 respiratory toxicity, 7 patients had metabolic toxicities grade 1–2. All patients engrafted. Patients achieved ANC > 500 on day 8–15 (median 12), platelets >25,000 on day 9 -22 (median 16), no RBC transfusions were required after day7–25, (median 16.2). Overall 1 ½ year survival rate is 87 % (11 out of 13 patients are alive); event free survival is 78%. Three patients had recurrent disease: 2 patients with PNET (both patients expired) and 1 pt with NBL (alive).

Conclusions: Analysis of the preliminary data suggests that B-M-T regimen in the context of HDT was tolerated without unexpected or severe toxicity and allowed prompt transition to radiation and/or maintenance therapy. Further studies are needed to document the survival benefit of consolidation with B-M-T regimen in children with high risk solid tumors.

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ALLOGENEIC STEM CELL IMMUNOTHERAPY FOR ADVANCED METASTATIC BREAST CANCER: THE WAY FORWARD

Blaise, D.[1], Goncalves, A.[1], Furst, S.[1], Bay, J.O.[2], Faucher, C.[1], Michallet, M.[3], Boiron, J.M.[4], Cahn, J.Y.[5], Gratecos, N.[6], Mohty, M.[7], Chabannon, C.[1], Gravis, G.[1], Esterni, B.[1], Extra, J.M.[1], Viens, P.[1] [1] Institut Paoli-Calmettes, Marseille, France; [2] Centre Jean Perrin, Clermont-Ferrand, France; [3] Hopital Edouard Herriot, Lyon, France; [4] EFS Aquitaine Limousin, Bordeaux, France; [5] CHU, Grenoble, France; [6] Hopital Archet 1, Nice, France; [7] Hotel Dieu, Nantes, France

Despite continuous advances in the treatment of mBcr, some patients have very poor outcome. Over the last period, we have investigated allogeneic immunotherapy as a possibility for tumor control in patients with advanced mBrC. We have treated 31 pts with Allo SCT in 2 successive clinical trials. All pts (age: 43 (27–57)) underwent ASCT after the same RIC (Fludarabin (150mg/m^2), Busulfan (8mg/kg) and Thymoglobulin (2,5mg/kg) or TLI (1 Cgy)) from a HLA-identical sibling (BM: 13%; PBSC: 87%) followed by CSA. Prior to ASCT a median of 3 lines of treatment (1–7) have been administered over a period of 57 months (6–143).15 (48%) pts underwent autologous SCT at a median time of 15 months (1–99) prior to ASCT. All pts were measurable and had a median of 2 metastatic sites (1–4) (liver:72%, bone:50%, lung:22% and brain:11%). At transplant : 17 (55%), 10 (32%) and 4 (13%) pts had progressive (PD), stable disease (SD) and partial response (PR) respectively. All patients engrafted. The cumulative incidences of grade 2–4 aGVHD and cGVHD were 42% (25–59) and 62% (45–79) respectively. Of note, none of the 31 pts died from TRM. Seven patients achieved an objective response (CR = 1; PR = 6) at a median of 60 days (30–150) for a 24 % (9–39) OR cumulative incidence. Eventually all pts but 3 progressed at a median of 310 days (120–560) post transplant. Four pts are alive at median of 23 months (21–30) post transplant for a 2-year overall survival (OS) probability of 29% (16–47)). Results are dramatically different in regards to disease status at time of transplant. While outcome was uniformly poor for pts with PD (OR = 0; 2 year OS probability: 6% (1–27)),